Long-term follow up of the CLL2007FMP trial evaluating fludarabine and cyclophosphamide in combination with either rituximab or alemtuzumab in previously untreated patients with chronic lymphocytic leukemia

In fit chronic lymphocytic leukemia (CLL) patients without chromosome 17p deletion (del(17p)) or TP53 mutation, six cycles of FCR (fludarabine, cyclophosphamide, rituximab) provides the best progression-free survival (PFS) and overall survival (OS).[1,2] In 2007, the French Innovative Leukemia Organization (FILO) group initiated the CLLFMP2007 study, a phase III trial in which fit patients with previously untreated CLL were randomized to six cycles of FCR or FCCam (fludarabine, cyclophosphamide, and alemtuzumab 30 mg subcutaneously on days 1-3 every 28 days). At the time, alemtuzumab, a humanized anti-CD52 monoclonal antibody, was one of the most active for treating CLL.[3] Recruitment onto the CLLFMP2007 study was prematurely stopped because of excess toxicity in the FCCam arm, including 8 deaths, 4 from lymphoma and 4 from infection, in this cohort of 165 patients. We reported the initial results in 2012 with a median follow up of 38 months.[4] Here, we provide the up-dated results with a median follow up of 76.4 months with particular attention to long-term outcome, toxicity, and minimal residual disease (MRD) data.

Treatment-naïve Binet stage B or C patients aged 18-65 years and without del(17p) were eligible for the study. Additional inclusion criteria were a cumulative illness rating scale (CIRS) score less than 7 and normal renal function. Patients were randomized 1:1 to six cycles of FCR or FCCam using *IGHV* mutational status and del(11q) as stratification factors. Baseline assessments included conventional karyotype and fluorescence *in situ* hybridization (FISH) analysis for del(13q), trisomy(12), del(11q), del(14q), and del(17p) and *IGHV* mutational status. MRD was assessed by 6-color flow cytometry in blood and bone marrow at month 9, and in blood at months 12 and 24. Follow up was performed every three months during the first year and every six months during the following two years; thereafter, patients were followed up annually for progression. Safety assessments included adverse events (AEs), serious adverse events (SAEs), clinical status, critical laboratory evaluations, and for patients treated in the FCCam group, monthly investigation for cytomegalovirus reactivation. For this analysis, the mutational status of *NOTCH/FBXW7, SF3B1, MYD88, XPO1, ATM, BIRC3* and *TP53* were determined by targeted DNA deep sequencing. A variant allele frequency minimal threshold of 5% was applied.

Analyses were performed as intent to treat. PFS was defined as the time between randomization and the first documented disease progression, death from any cause, or last follow up for surviving patients. PFS and OS were estimated by the non-parametric Kaplan-Meier method and then compared between randomized groups by the log-rank test. Treatment comparisons were adjusted for imbalances or prognostic covariates using a multivariable Cox model. Binary outcomes were crudely compared between randomized groups with the Fisher exact test and then adjusted for prognostic covariates using the logistic regression model. Statistical analyses were performed using SAS v.9.2 (SAS Institute).

The study included 165 patients without deletion 17p. Patients' characteristics were similar between the two groups. Most patients were male (73%), and the median age was 57 years. Eighty percent of patients had Binet stage B disease. Most patients had unmutated *IGHV* and lacked del(11p), trisomy(12), and 14q32 rearrangement. About half were positive for del(13q) (Table 1). After a median follow up of 76.4 months, 36 events had occurred in the FCR arm (33 progressions and 3 deaths without relapse) and 34 in the FCCam arm (27 progressions and 7 deaths without relapse) (P=0.57). The probability of PFS was not significantly different between the study arms [64.5% (95%CI: 54.6-76.2) for FCCam *vs.* 60.0% (95% CI: 50.2-71.8) for FCR; P=0.57] (Figure 1). The probability of OS was also not significantly different between the study arms [75.3% (95%CI: 66.4-85.3) for FCCam *vs.* 85.2% (95%CI: 77.8-93.3) for FCR; P=0.11]. Female sex, Binet stage C, and elevated β2-microglobulin were independent predictors of a shorter OS. Binet stage C, unmutated IgVH status, and XPO1 mutation were independent predictors of a shorter PFS. In addition, undetectable MRD in the peripheral blood (defined as < 1 CLL cell per 10,000 leukocytes) at month 9 was highly predictive of a longer PFS (P<0.0001) but not a longer OS (P=0.76) (Figure 2). Undetectable MRD at months 12 and 48 were similarly predictive of a longer PFS but not of a longer OS.

Since the initial report,[4] 9 new SAEs (all infectious complications) were reported in the FCCam arm and 4 new SAEs (one infectious toxicity and 3 secondary neoplasias) were reported in the FCR arm. Since initiation of the study, a total of 32 patients have died: 20 in the FCCam

Table 1. Mutation status at baseline.

Parameter	n (%) FCCam	FCR
IGHV status	N=83	N=82
Mutated	33 (39.8)	37 (45.1)
Unmutated	50 (60.2)	45 (54.9)
Del(11q)	N=83	N=82
Yes	17 (20.5)	16 (19.5)
No	66 (79.5)	66 (80.5)
Del(13q)	N=79	N=77
Yes	43 (54.4)	40 (51.9)
No	36 (45.6)	37 (48.1)
Trisomy(12)	N=79	N=77
Yes	10 (12.9)	13 (16.8)
No	69 (77.1)	64 (83.2)
14q32 rearrangement	N=71	N=72
Yes	12 (16.9)	12 (16.7)
No	59 (83.1)	60 (83.3)
Karyotype	N=61	N=62
Complex	7 (11.5)	6 (9.7)
Mutations	N=71	N=72
ATM1	9 (12.7)	10 (13.9)
BIRC3	4 (5.6)	3 (4.2)
FBXW7	2 (2.8)	5 (6.9)
MYD88	1 (1.4)	5 (6.9)
NOTCH1	8 (11.3)	16 (22.2)
SF3B1	12 (16.9)	18 (25.0)
TP53	2 (2.8)	5 (6.9)
XPO1	8 (11.3)	7 (9.7)

arm and 12 in the FCR arm (P=0.11). The most common causes of death were infections (n=6) and disease progression (n=6) in the FCCam arm and progression (n=6) in the FCR arm. In this up-dated analysis, and after a median follow up of 76.4 months, PFS was not longer in the FCR arm than in the FCCam arm, despite excess of toxicity in the FCCam arm. There was no difference in OS, although patients mainly died from progression of CLL in the FCR arm. This longer follow up did not reveal additional toxicities because most had occurred within the first two years. FCR remains the first-line treatment of choice for fit CLL patients without *TP53* alterations.

Addition of alemtuzumab to FC did not improve survival and lead to excess of toxicity. Alemtuzumab has been used in 2 prospective trials but with a low dose approach (30 mg per cycle). In relapsed patients, median PFS was two years and myelosuppression was the most common AE; Authors suggested a close vigilance of

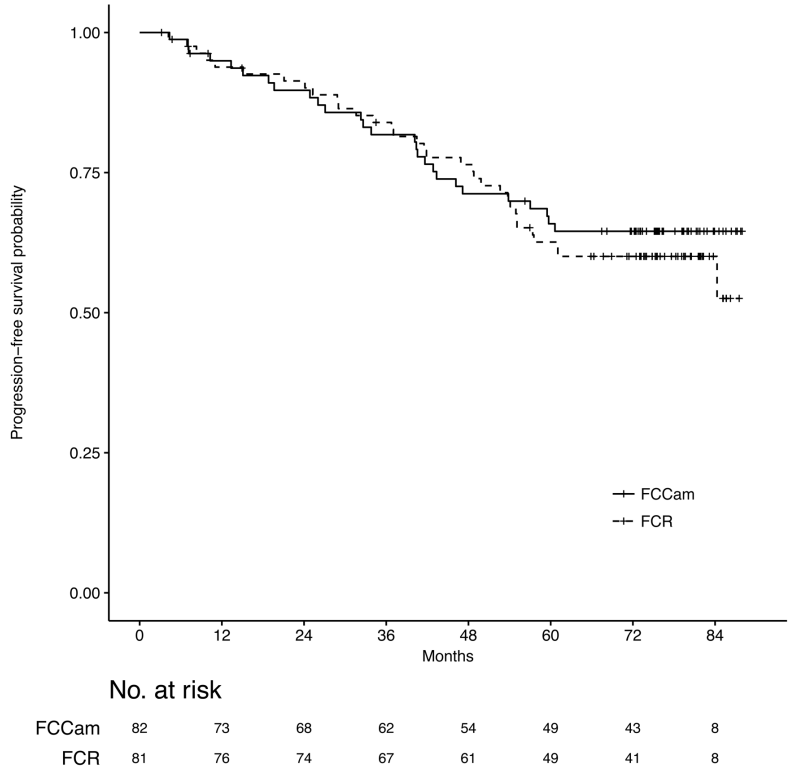

Figure 1. Long-term progression-free survival survival (PFS) in the FCCam (dashed line) and FCR (solid line) arms of the study. PFS was estimated for the FCCam and FCR arms by the non-parametric Kaplan-Meier method and compared by log-rank test.

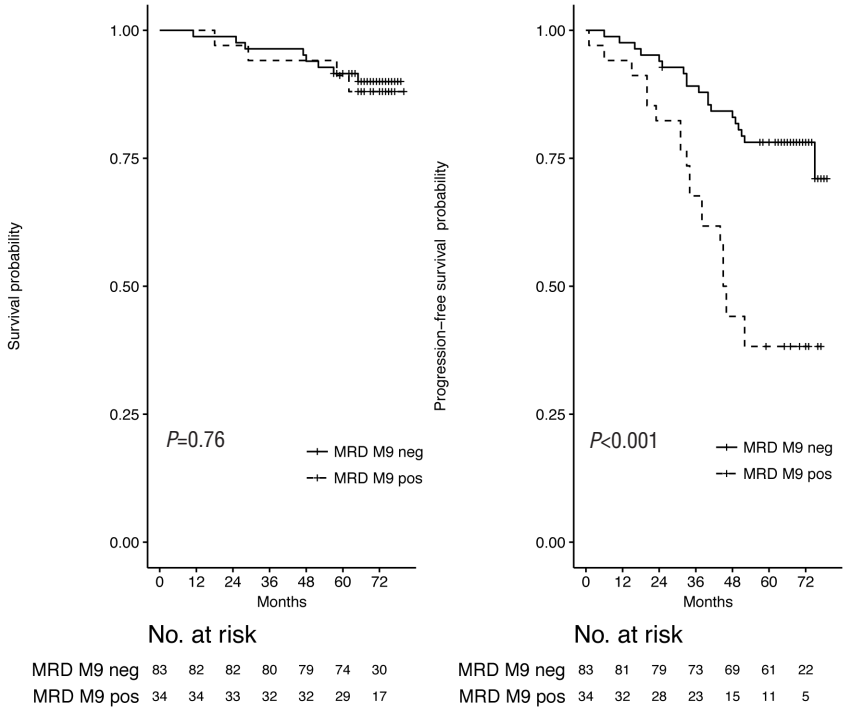

Figure 2. Overall survival (OS) and progression-free survival (PFS) according to the presence of minimal residual disease (MRD) in the peripheral blood at month 9. PFS and OS were estimated for patients negative (MRD neg: solid line) and positive (MRD pos: dashed line) for MRD at month 9 (i.e. 3 months after treatment completion) by the non-parametric Kaplan-Meier method and compared by log-rank test.

opportunistic infections.[5] In a phase III trial comparing FCCam to FC in first line, FCCam prolongs 3-year PFS (53% *vs.* 37%) without a higher mortality rate.[6,7] This low-dose regimen seems to be less toxic but less effective than FCR. In the CLL8 trial comparing FCR *versus* FC treatment, median PFS was 56.8 months, although 6% had del(17p).[8] Our analysis confirmed these data; after a median follow up of 76.4 months, the probability of PFS was 60%. Deletion of chromosome 11, *TP53* mutation, and other recently described mutations including *NOTCH1, SF3B1*, and BIRC39,[10] were not, however, predictive of outcome in our series. In the case of *TP53* mutation, this may have been due to fact that it was present only in a few patients as patients with del(17p) were excluded.

Eradicating MRD has been proposed as a goal in CLL treatment.[11] Indeed, we found that undetectable MRD in the peripheral blood at month 9, 12, or 48 was an independent prognostic indicator for longer PFS, irrespective of the treatment arm, type of response, or pre-treatment risk factors, which supports MRD as a treatment goal. However, undetectable MRD in the peripheral blood was not predictive of better OS. This could have been due to a significant effect of the site of MRD sampling; when sampled early after treatment completion, bone marrow is considered a more sensitive site for detecting MRD than blood.[12] Unfortunately, the number of bone marrow samples was too low to reach significance.

In conclusion, long-term follow up of the CLL2007 trial confirmed that FCCam does not provide a better outcome than FCR but is more toxic. This trial supported the idea that MRD measured three months after treatment completion can be used to predict outcome. Adapting first-line treatment according to MRD response is currently being evaluated in a first-line trial by the FILO group.

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